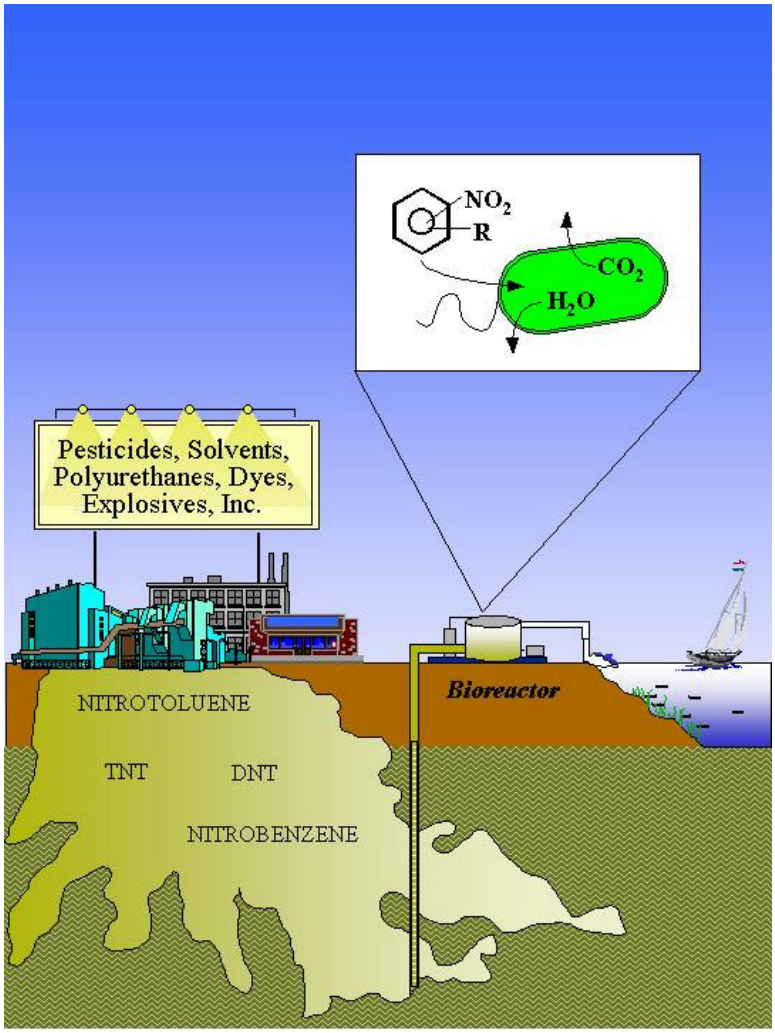
TECHNOLOGY STATUS REVIEW: BIOREMEDIATION OF DINITROTOLUENE (DNT)



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Technology Status Review: Bioremediation of Dinitrotoluene (DNT)

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Purpose

Recent advances in the understanding of how bacteria biodegrade dinitrotoluenes (DNT) under aerobic conditions has led to the development of remediation systems that can dramatically reduce clean up costs of DNT-contaminated soil and ground water. This document summarizes the latest information on bioremediation technologies that exploit the ability of aerobic bacteria to mineralize 2,4- and 2,6-dinitrotoluene (2,4-DNT, 2,6-DNT) to yield energy, harmless minerals and biomass (1). It is based on a recent review of the relevant literature (8). Sources for further information are provided below.

Background

Biodegradation can result in either mineralization or transformation of DNT. Mineralization, the complete catabolism of a compound to its inorganic components is the preferred goal of bioremediation systems (2). Energy derived from the catabolic process provides a selective advantage to the degradative organism. Transformation (cometabolism) (1) is much less desirable for several reasons. The requirement for a primary substrate and the absence of a selective advantage renders cometabolic systems more expensive and difficult to control than systems that rely on mineralization. Transformation also produces organic derivatives of the parent molecule whose identity and toxicity must be established for each individual situation. Transformation of DNT leads to partial reduction and formation of amines.

Both mono- and dinitrotoluenes are susceptible to aerobic microbial degradation, and the catabolic pathways are known (8). Bacteria that grow on the predominant DNT isomers as sole carbon, nitrogen and energy source have been isolated from contaminated systems worldwide (7-9, 13). In a few instances the genes that encode the degradative enzymes have been cloned and sequenced. Given the knowledge of the degradative pathways and the physiological requirements of the degradative microorganisms, systems can be designed to meet the growth requirements of the organisms and to control or monitor the catabolic process.

Productive anaerobic pathways for degradation of mono- and dinitrotoluenes are not known. However, cometabolic reduction of the nitro group occurs under both anaerobic and aerobic conditions. The reductive transformations are attributed to the activity of non-specific nitroreductases (3). The cultures are generally grown with simple sugars or alcohols to provide growth substrates and electron donors. In studies in which the products of anaerobic bacterial reduction of 2,4-DNT were carefully analyzed (5, 6), nitroso-, aminonitro-, and diamino-compounds predominated. Cometabolic reduction and acetylation of 2,4-DNT has also recently been demonstrated under aerobic conditions as well as anaerobic conditions in *Pseudomonas aeruginosa* cultures (10). In general, non-specific reduction does not lead to ring cleavage and further transformation of the metabolites. Thus aerobic treatment seems much more effective.

Applicability and Limitations

Mineralization of mixtures of 2,4-DNT and 2,6-DNT has been demonstrated at bench-, pilot- and field-scale in a variety of soil and ground water systems, both in situ and ex situ. The presence of specific DNT-degrading bacteria at sites that are chronically contaminated with DNT raises the possibility of natural attenuation as a DNT remediation alternative. Biodegradation of DNT will generally occur under the following conditions:

1. O_2 concentration > 1 mg/L
2. Adequate and stable moisture
3. pH between 6.5 and 8.5
4. Moderate soil/water temperatures
5. Adequate macronutrients (phosphate, sulfate)
6. Appropriate bacterial biomass

There are a number of factors that must be considered before bioremediation of DNT can be used. DNT degradation is negligible under anaerobic conditions. When more readily degradable carbon sources such as simple alcohols or sugars are present, cometabolic transformation rather than mineralization will be the predominant microbial process. If an in situ remedy is under consideration, the finding that indigenous bacteria will degrade rather than transform DNT indicates that cometabolic transformations will not be substantial as long as conditions are aerobic. A large excess of 2,4-DNT over 2,6-DNT can prevent simultaneous degradation of the two isomers necessitating a sequential system to fully degrade 2,6-DNT. The low C/N ratio of the DNT molecule can result in the accumulation of excess nitrite that lowers the pH of the system, and can pose disposal problems if the initial DNT concentrations are high. Ex situ bioremediation

allows a high degree of control of the significant environmental variables. Recent experience (discussed below) has shown that the limitations discussed above can be managed in appropriately engineered ex situ treatment systems. In situ treatment, though far less expensive, offers less control of the key limiting factors, and therefore more careful analysis is required early in the design process to ensure the effectiveness of the treatment system.

Examples

Fluid bed reactors for ground water remediation

The Volunteer Army Ammunition Plant (VAAP) near Chattanooga, TN was a TNT manufacturing plant from 1941 until 1977 (14). DNT contamination of soil and ground water is quite heterogeneous and concentrations of nitrotoluenes in ground water vary with rainfall. Studies conducted with contaminated ground water from VAAP revealed how mixtures of mono- and dinitrotoluenes are degraded in fluidized bed reactors (FBR) at bench- and pilot-scale.

A preliminary demonstration was performed in a bench-scale FBR inoculated with a mixed culture of DNT-degrading strains (4, 12). Removal efficiencies for DNT were greater than 98% at hydraulic retention times greater than 1.5 h. The study yielded insight about the configurations and limitations of FBRs for DNT degradation. The retention of the induced biomass was critical to the success of the system. With a large induced biomass and complete mixing of the biomass and feed, the concentration of DNT within the reactor vessel was always at a very low level. Therefore, the two DNT isomers were degraded simultaneously with no apparent inhibition by either isomer. When ground water containing 2-NT and 4-NT in addition to 2,4- and 2,6-DNT was used as the feed, acclimation of the biomass to degrade the mononitotoluenes required 10 days. The bench-scale studies demonstrated the feasibility of simultaneously degrading mixtures of mono- and dinitrotoluenes at high rates.

A pilot-scale field study was conducted based upon the parameters established by the laboratory-scale FBR. The reactor contained a granular activated carbon biocarrier inoculated with a mixed culture of 2,4-DNT, 2,6-DNT, 2-NT and 4-NT-degrading strains (14). The removal efficiencies for 2,4-DNT, 2-NT, and 4-NT were always high, and removal of TNT fluctuated in a narrow range around 50% depending on hydraulic retention time. 2,6-DNT removal started slowly but there was a dramatic improvement after 4 months of operation. During the time when 2,6-DNT was degraded poorly in the primary reactor, it could be degraded effectively in a subsequent extended aeration vessel. The fluidized bed reactor technology is a more cost effective treatment method for DNT than either UV/ozone treatment or liquid phase granular activated carbon adsorption when the total nitrotoluene concentrations are relatively high. Recently developed strategies to improve the efficiency of 2,6-DNT degradation can improve the economies considerably.

Soil slurry reactors

Studies with field contaminated soils showed that DNT contamination over 50 years old can be effectively removed from soil by degradative bacteria (9). Bench-scale experiments demonstrated that bioremediation of DNT in aged field-contaminated soils was rapid and extensive. Pilot-scale studies have established the reactor configurations and operational conditions for scale-up of slurry reactor systems for the treatment of DNT-contaminated soils (16). Airlift bioreactors (Eimco 70-L) were used to treat DNT contaminated soils from VAAP and from the Badger Army Ammunition Plant (BAAP) near Baraboo, WI. The BAAP soil contained 2,4-DNT (11 g/kg) and 2,6-DNT (0.2 g/kg). The VAAP soil had similar levels of 2,4-DNT, but higher 2,6-DNT concentrations (1 g/kg) and significant amounts of TNT (0.4 g/kg). Degradation of 2,4-DNT was rapid, predictable and easily established for both soils at initial concentrations up to 11.2 mM (2.0 g/L) 2,4-DNT (Fig. 1). At concentrations > 5 mM (0.9 g/L), 2,4-DNT was degraded at 0.9 – 1.4 g/L/d. At concentrations < 2 mM (0.36 g/L), the rate ranged from 0.2 – 0.3 g/L/d. Nitrite toxicity became a problem at very high soil loading levels, and limited the soil loading rate.

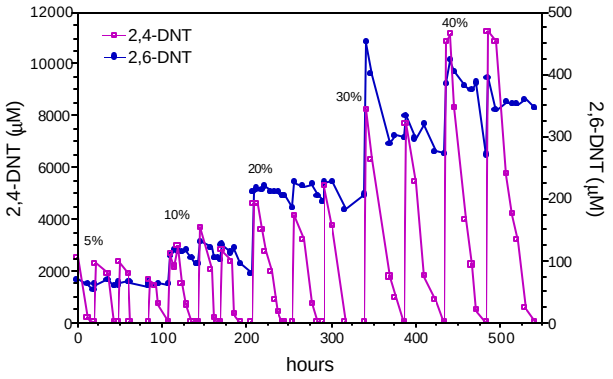

Fig. 1. Slurry-phase concentrations of DNT in Eimco reactor fed BAAP soil at 5, 10, 20, 30, and 40% nominal soils loading rates. [Modified from (16)]

Initially, 2,6-DNT was not degraded in the bioreactors. Shake flask studies showed that high ratios of 2,4-DNT to 2,6-DNT inhibited 2,6-DNT degradation. The problem was overcome by conducting the 2,6-DNT degradation phase in a separate reactor placed in series. After separation of the two degradation processes and an acclimation period, 2,6-DNT was degraded efficiently (Fig. 2). At concentrations between 150 and 300 µM, 2,6-DNT was degraded at 0.11 – 0.29 g/L/d. Low residual

concentrations of DNT remained in the treated slurry following both single and sequential reactor treatment, but the sequential treatment reduced the residuals to below the EPA treatment standard limits (40 CFR 268.48). The 2,6-DNT-degrading bacteria were much more tolerant of nitrite accumulation than the 2,4-DNT-degrading cultures and the high nitrite levels that were carried over from the 2,4-DNT reactors into the 2,6-DNT reactors had no effect on 2,6-DNT degradation. It is clear that 2,4-DNT inhibits 2,6-DNT degradation, but the mechanism is not known. Separation of the two operations can enhance the efficiency of the overall bioremediation system. The bench-scale and pilot-scale experiments indicate that inoculation with specific DNT-degrading bacteria can hasten the development of a stable DNT-degrading population even in the presence of an indigenous population. Inoculation is particularly valuable for 2,6-DNT degradation where long acclimation periods appear to be the norm.

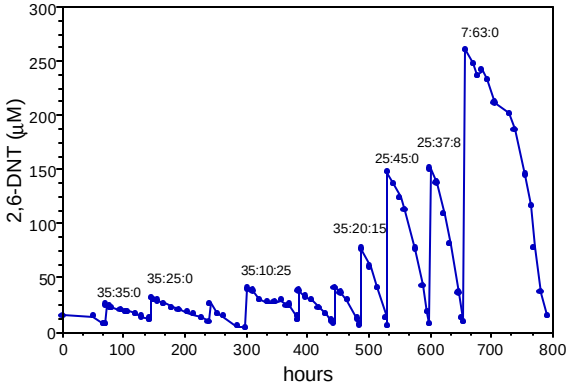

Fig. 2. Concentration of 2,6-DNT in reactor fed effluent from reactor in Fig. 1. Ratios denote volume remaining from previous cycle, volume of fresh effluent added, volume of tap water diluent. [Modified from (16)]

A small residual fraction of the DNT can persist in the treated solids when DNT is no longer detectable in the aqueous phase. Field-contaminated soils, but not the laboratory-contaminated soil or artificially-contaminated clay aggregates (11), retain low levels of acetonitrile-extractable DNT after treatment. The residual DNT seems not to be bioavailable or extractable with water. Treatment of DNT-contaminated soil in bench-scale bioreactors greatly reduced the toxicity (9); however, similar tests should be conducted to determine whether the residual DNT in soils from bioremediation systems requires further treatment. About half of the initial TNT in the VAAP soil disappeared during the bioreactor treatment. A small amount of the missing TNT was detected as aminodinitrotoluene. Strategies to remove or immobilize residual TNT and TNT metabolites should be incorporated into the overall remediation plan when soils contain both DNT and TNT.

In situ applications

The discovery of bacteria in the environment that are able to degrade nitroaromatic compounds strongly suggests that natural attenuation or other in situ processes can be suitable remediation strategies for nitroaromatic contaminants. At BAAP, waste materials from the reprocessing of single-base propellants were deposited into large in-ground waste pits. Six waste pits, each roughly 40 feet in diameter and extending 100 feet down to the water table contain soil heavily contaminated with 2,4-DNT (15). One of the waste pits is the source of a DNT-contaminated ground water plume. DNT concentrations in the ground water plume decrease in downgradient wells. 2,3-Dinitrotoluene (2,3-DNT) does not decrease at the same rate as 2,4- and 2,6-DNT. 2,3-DNT has not been demonstrated to be biodegradable and can thus be considered a conservative tracer that reflects the effects of abiotic processes. The much greater decrease in 2,4- and 2,6-DNT concentrations can therefore be attributed to biological activity. 2,4-DNT-degrading bacteria have been isolated from monitoring well water from the site and 2,4-DNT disappears with stoichiometric release of nitrite from microcosms constructed with DNT-contaminated soil from the site. The understanding of the 2,4-DNT catabolic pathway taken with laboratory studies with soil from the site, disappearance of DNT from the monitoring wells, and the isolation of bacteria able to degrade DNT from the same wells, provide evidence that natural attenuation is taking place at the site. Stoichiometric release of nitrite demonstrates complete mineralization of DNT which precludes formation of significant amounts of amino compounds. Similar results have been obtained from two industrial sites contaminated with DNT.

DNT contamination in the vadose zone at BAAP where 2,4-DNT occurs at concentrations up to 28% by weight (15), is currently being treated by in situ bioremediation. A pilot-scale treatment system, designed by Stone & under the direction of the U.S. Army Corps of Engineers, is based on the results of bench-scale treatability tests in which ground water was recirculated through contaminated soil columns. In the pilot-scale system, ground water from the bottom of one of the waste pits is reintroduced to the top of the waste pit through an infiltration gallery just below ground level. Air is introduced via sparge wells in the waste pit. Approximately 75% of the water is recirculated. Nitrite released by DNT degradation is removed by denitrification in an anoxic reduction zone established down gradient. Preliminary results from the pilot system demonstrate that: 1) 2,4-DNT is rapidly degraded in situ, 2) acclimation following inoculation with indigenous microorganisms is rapid, 3) the system is very stable

and robust, 4) nitrite is oxidized to nitrate by indigenous bacteria which limits the accumulation of toxic levels of nitrite, and 5) no cosubstrates are required for DNT degradation. Based on early results of the pilot-scale treatment system, the Army Corps of Engineers has approved the design and implementation of a full-scale treatment system. More information about work at BAAP and other related Army sites can be found at http://www.badgeraap.org/index.shtml.

Further Considerations

The studies cited above raise a few issues that require further consideration. The first is that simultaneous degradation of 2,4-DNT and 2,6-DNT is unpredictable. However, sequential degradation of the two isomers is reliable once an adapted population is established. Second, accumulation of nitrite/nitrate must be considered both to meet regulatory standards for any effluents generated, and to prevent inhibition of DNT degradation by nitrite accumulation. The oxidation of nitrite to nitrate is unpredictable and has only been observed in two instances during numerous laboratory studies and one field study. Third, the distribution of degradative bacteria must be considered, particularly if in situ strategies are under consideration. Nitrotoluene-degrading bacteria are not ubiquitous, and neither the mechanism nor the time course of the evolution or distribution of such bacteria are currently understood. To date they have been found at most contaminated sites, but the presence of bacteria with the ability to degrade DNT must be verified at each site. Fourth, the endpoints for bioremediation of DNT are not well established. And fifth, TNT is not substantially degraded during aerobic treatment of DNT.

Summary

Bioremediation can be an effective method for treating DNT-contaminated soil and ground water, and is less costly than competing accepted technologies. 2,4-DNT is more easily degraded than 2,6-DNT, and sequential treatment systems may be needed to treat soil or water containing both isomers. TNT is far less biodegradable than these DNT isomers, and the presence of TNT may make bioremediation more difficult or expensive. 2,3-DNT is apparently not biodegradable.

Site-specific laboratory testing is essential prior to selection and design of a bioremediation system. Key issues for laboratory tests include: 1) appropriate chemical and physical conditions (pH, redox, nutrients); 2) verification of the presence of bacteria capable of DNT mineralization; 3) presence of indigenous nitrite oxidizing bacteria; and 4) whether achievable endpoints are acceptable in terms of toxicity and risk. Laboratory tests may also be needed to estimate DNT degradation rates, to establish maximum DNT concentrations that can be biodegraded, and to design strategies to biodegrade mixtures of DNT isomers and/or mononitrotoluenes.

In situ bioremediation is possible at sites where: 1) aerobic conditions are present or can be engineered; 2) appropriate organisms are present or can be introduced effectively; 3) the potential for nitrite or nitrate accumulation can be managed. Ex situ bioremediation is more expensive but may be needed at sites that do not meet the above criteria. Fluidized bed reactors have been demonstrated for treatment of DNT in water, and soil slurry reactors are effective for contaminated soils. In either case, significant effort must be spent on the engineering design to ensure that DNT treatment will be successful and cost-effective, particularly when mixtures of 2,4-DNT and 2,6-DNT are present.

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